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PROCESS FOR THE PREPARATION OF PHOSPHITYLATION AGENTS

The present invention concerns a process for the preparation of phosphitylation agents.

Synthetic oligonucleotides are important diagnostic tools for the detection of genetic and viral diseases. In addition, oligonucleotides and modified oligonucleotides are of interest as therapeutic candidates that inhibit gene expression or protein function. Large scale synthesis of oligonucleotides for use as therapeutic candidates has become increasingly important since FDA approval of an oligonucleotide analog for the treatment of cytomegalovirus (CMV), and several other oligonucleotide analogs are currently in clinical trials. Kilogram quantities of a purified oligonucleotide analog are needed for each clinical trial.

The principal method currently employed for the preparation of oligonucleotide is the phosphoramidite approach. The increasing demand for larger quantities of oligonucleotides has correspondingly increased demand for phosphoramidite compounds. Phosphoramidite compounds are commonly prepared by phosphitylation of a nucleoside with a phosphitylation agent in the presence of an activator. Accordingly, there has been a corresponding increase in demand for phosphitylation agents. Improved or alternative processes for the preparation of phosphitylation agents are therefore needed.

According to the present invention, there is provided a process for the preparation of a compound of formula $R^1\text{-}Y^1\text{-}P(NR^2R^3)_2$ which comprises:

a) reacting a compound of formula PX_3 with a compound of formula HNR^2R^3 to form a compound of formula $X\text{-}P(NR^2R^3)_2$; and

b) reacting the compound of formula $X\text{-}P(NR^2R^3)_2$ with a compound of formula $R^1\text{-}Y^1\text{-}H$ in the presence of a solvent to form the compound of formula $R^1\text{-}Y^1\text{-}P(NR^2R^3)_2$

wherein

R^1 represents a phosphorus protecting group;

R^2 and R^3 each independently represent an alkyl, preferably a C_{1-6} alkyl, group, or R^2 and R^3 are joined, together with the N to which they are attached, to form a 5-7 membered ring;

Y^1 represents O or S, preferably O; and

X represents a halogen, preferably Cl;

characterised in that the solvent employed in reaction b) is a hydrocarbon solvent.

Phosphorus protecting groups represented by R^1 are commonly cleavable phosphorus protecting groups employed in oligonucleotide synthesis, for example substituted or unsubstituted aliphatic groups, such as a methyl group, $-CH_2CH_2-Si(CH_3)_2C_6H_5$, $-CH_2CH_2-S(O)_2-CH_2CH_3$, $-CH_2CH_2-C_6H_4-NO_2$ and preferably a group of formula $-CH_2CH_2CN$; or substituted or unsubstituted aromatic groups, such as a phenyl or

substituted phenyl, for example a 4-chlorophenyl, 2-chlorophenyl, 2-nitrophenyl or 4-nitrophenyl group.

Compounds of formula R^1-Y^1-H are preferably selected based on the nature of the compound it is desired to produce. An especially preferred compound of formula R^1-Y^1-H is 2-cyanoethanol.

In the compounds prepared by the process of the present invention, it is preferred that R^2 and R^3 are the same. It is particularly preferred that both R^2 and R^3 are $-CH(CH_3)_2$ groups. It is especially preferred that Y^1 is O and R^1 is $-CH_2CH_2CN$.

Examples of compounds which can be prepared by the process of the present invention include O-β-cyanoethyl-N,N,N',N'-tetraisopropylphosphorodiamidite, (commonly known as "tetraphos"), O-β-cyanoethyl-N,N,N',N'-tetramethylphosphorodiamidite, O-β-cyanoethyl-N,N,N',N'-tetraethylphosphorodiamidite, bis (N,N-diisopropylamino)-2-methyltrifluoroacetylamino-ethoxyphosphine, bis (N,N-diisopropylamino)-2-diphenylmethylsilylethoxyphosphine and O-β-cyanoethyl-bis (N-morpholino) phosphorodiamidite.

Hydrocarbon solvents that can be employed in the process of the present invention include aliphatic and aromatic hydrocarbons. Examples of aliphatic hydrocarbons include pentane, hexane and petroleum ethers. Examples of aromatic hydrocarbons include benzene, toluene, xylene and mesitylene. Toluene is the most preferred solvent.

In many preferred embodiments, reaction a) takes place in the presence of the same solvent as reaction b), and most preferably in the presence of toluene.

The reaction between the compound of formula $X-P(NR^2R^3)_2$ and the compound of formula R^1-Y^1-H preferably takes place in the presence of a base. Bases which can be employed include inorganic bases, such as sodium carbonate, and organic bases. Organic bases are preferred. Examples of organic bases include aromatic amines such as pyridine, and inorganic amines, such as alkylamines, preferably trialkylamines, such as tri(C_{1-4}alkyl)amines, and most preferably triethylamine.

The mole ratio of compound of formula PX_3 to compound of formula HNR^2R^3 in step a) is commonly selected to be in the range of from about 1 : 1 to about 10 : 1, and preferably from about 3 : 1 to about 6 : 1.

The mole ratio of compound of formula $X-P(NR^2R^3)_2$ to compound of formula R^1-Y^1-H in step b) is commonly selected to be in the range of from about 1 : 1 to about 5 : 1, with mole ratios in the range of from 1 : 1 to 1.5 : 1 being especially preferred.

When a base is employed, the mole ratio of base to compound of formula $X-P(NR^2R^3)_2$ is often in the range of from about 0.75 : 1 to 2 : 1, and preferably from about 1 : 1 to 1.3 : 1.

Step a) of the process according to the present invention is often carried out at a temperature in the range of from ambient temperature, such as from about 15°C to about

30°C, up to the reflux temperature of the solvent employed, such as from about 50°C to about 120°C.

Step b) of the process according to the present invention is often carried out at a temperature in the range of from about -25°C to ambient temperature, such as from about 15°C to about 30°C, such as from about -20°C to about 0°C. Temperatures in the range of from -20°C to -10°C are especially preferred.

Advantageously, substantially anhydrous reaction conditions are employed.

In many embodiments the process of the present invention is carried out under an inert atmosphere, such as a nitrogen or argon atmosphere.

The product compound of formula R^1-Y^1-$P(NR^2R^3)_2$ is advantageously separated from the reaction mixture by distillation, and especially preferably by wiped-film distillation.

A particularly preferred embodiment of the present invention comprises a process for the preparation of $\{[(CH_3)_2CH]_2N\}_2$-P-O-CH_2CH_2CN, which comprises

a) reacting PCl_3 with $[(CH_3)_2CH]_2N$-H in toluene to form $\{[(CH_3)_2CH]_2N\}_2$-P-Cl; and

b) reacting $\{[(CH_3)_2CH]_2N\}_2$-P-Cl with HO-CH_2CH_2CN in toluene to form $\{[(CH_3)_2CH]_2N\}_2$-P-O-CH_2CH_2CN.

The present invention is illustrated without limitation by the following Example.

Example

Step a)

Diisopropylamine (383g), toluene (1087g) and calcium hydride (10g) were charged to a nitrogen-flushed, 2 L round-bottom flask equipped with a magnetic stirrer and cold-finger distillation head attached to an N_2 bubble and the mixture heated under total reflux (98°C) for 2 hrs to remove all traces of water. The dried amine/toluene mixture was distilled into an oven-dried nitrogen-flushed, 3L, 4-neck round-bottom flask equipped with a mechanical stirrer, a 100 mL, pressure-equalizing addition funnel, a thermowell and a condenser attached to an N_2 bubbler, and allowed to cool to ambient temperature (ca. 17°C). Phosphorus trichloride (99.999%, 100g) was charged from the addition funnel over approximately 30 min. The temperature of the mixture was observed to rise ca. 25°C. The mixture was heated to reflux (100-110°C) and stirred for 24 hrs; the mixture becoming quite thick with precipitated diisopropylamine hydrochloride. The mixture was cooled to ambient temperature and pressure-filtered under nitrogen to remove the amine salt, all equipment being oven-dried before use. The filter cake was washed with 217g of toluene, and the combined filtrate and washings distilled *in vacuo* to remove most of the toluene, the mixture becoming a slush as solids precipitate. When no more toluene distilled, the vacuum was broken with nitrogen and the distillation vessel connected via a cold trap directly to a vacuum pump. Residual toluene was pumped out until the solid product, $\{[(CH_3)_2CH]_2N\}_2$-P-Cl, reached constant weight. This material, 96-97 area-% active by ^{31}P NMR was used in Step b) without further purification.

Step b)

Triethylamine (49.4g), toluene (649.5g) and calcium hydride (5g) were charged to a nitrogen-flushed, 1L round-bottom flask equipped with a magnetic stirrer and cold-finger distillation head attached to an N_2 bubbler and the mixture heated under total reflux (98°C) for 2 hrs to remove all traces of water. The dried mixture was distilled into an oven-dried, nitrogen-flushed, 2L 3-neck, jacketed round-bottom flask equipped with a mechanical stirrer, a thermowell and a 50 mL pressure-equalizing addition funnel attached to an N_2 bubbler and the mixture cooled to below -15°C using a circulating chiller pumping ethylene glycol/water through the reactor jacket and the product of step (a) (130.3g) added. Neat 2-cyanoethanol (39.9g) was added from the addition funnel over approximately 30 min, keeping the temperature below -10°C. The mixture was stirred under nitrogen for 18 hrs at -15 to -18°C to complete the reaction. The mixture was warmed to ambient temperature and pressure-filtered under nitrogen to remove triethylamine hydrochloride salt, all equipment being oven-dried before use. The toluene was stripped out on a rotary evaporator (bath temperature ca. 50°C). The vacuum was broken with nitrogen and the distillation vessel connected via a cold trap directly to a vacuum pump and residual toluene removed to yield 139.5g of crude product. The crude product was distilled through a wiped-film evaporator (heated zone 70°C; pressure 0.008 mmHg) over 1.5 hrs to afford 115.9g of product.

If desired, the product can be further purified by flash chromatography, for example using pentane and dry basic alumina, and additional wiped-film distillations.